(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 May 2003 (30.05.2003)

PCT

(10) International Publication Number
WO 03/044058 A2

(51) International Patent Classification[7]: C07K 16/40

(21) International Application Number: PCT/EP02/13074

(22) International Filing Date:
21 November 2002 (21.11.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
01870260.5 23 November 2001 (23.11.2001) EP

(71) Applicant *(for all designated States except US)*: **UNIVERSITE CATHOLIQUE DE LOUVAIN** [BE/BE]; 1 Place de l'Université, B-1348 Louvain-la-Neuve (BE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **EECKHOUT, Yves, Joseph** [BE/BE]; 38 avenue de l'Espinette, B-1348 Louvain-la-Neuve (BE). **COURTOY, Pierre, Joseph** [BE/BE]; 4 rue Pierre Delacroix, B-1150 Bruxelles (BE). **MARBAIX, Etienne, Michel** [BE/BE]; 2 Clos du Nysdam, B-1310 La Hulpe (BE).

(74) Agents: **BRANTS, Johan, Philippe, Emile** et al.; De Clercq, Brants & Partners, E. Gevaertdreef 10 a, B-9830 Sint-Martens-Latem (BE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/044058 A2

(54) Title: MEDICAL USE OF ANTIBODIES DIRECTED AGAINST HUMAN MATRIX METALLOPROTEINASES OR RELATED TISSUE PROTEINASES FOR THE TREATMENT OF ABNORMAL UTERINE BLEEDING AND ENDOMETRIOSIS

(57) Abstract: The invention relates to the field of metalloproteinases and their involvement in abnormal uterine bleeding or in endometriosis. The invention provides inhibitors of tissue proteinases or of metalloproteinases for preparing medicaments for treating or preventing bleeding disorders of the endometrium. More specific, these inhibitors are inhibitory antibodies against matrix metalloproteinases.

MEDICAL USE OF ANTIBODIES DIRECTED AGAINST HUMAN MATRIX METALLOPROTEINASES OR RELATED TISSUE PROTEINASES FOR THE TREATMENT OF ABNORMAL UTERINE BLEEDING AND ENDOMETRIOSIS

FIELD OF THE INVENTION

The invention relates to the field of metalloproteinases and their involvement in abnormal uterine bleeding or in endometriosis.

BACKGROUND OF THE INVENTION

Abnormal uterine bleeding is a common disorder requiring frequently surgical interventions such as hysterectomy or endometrial resection and/or ablation. It comprises excessive or prolonged menstrual bleeding (menorrhagia), bleeding outside menstrual periods in the absence (metrorrhagia) or in the presence of hormonal treatment (breakthrough bleeding). For example, in patients upon progestin-only contraception, irregular bleeding without organic lesion is particularly frequent, causing treatment discontinuation in about 25 % of women, thereby impeding the control of human population in developing countries. In addition, bleeding of ectopic endometrial tissue represents a major concern in the therapy of endometriosis. For most women with pathological bleeding, no efficient pharmacological options are presently available. In the US, 30 to 45 % of the hysterectomies are performed for abnormal uterine bleeding, i.e. between 228000 and 292000 hysterectomies each year.

Therefore it is an aim of the present invention to provide new medicaments and new therapies for abnormal uterine bleeding and endometriosis.

DESCRIPTION OF THE INVENTION

The present inventors have shown that normal and abnormal uterine and endometriotic bleedings are triggered by the expression and activation of matrix metalloproteinases (MMPs) or related proteinases. In particular MMP inhibitors are capable of blocking menstrual lysis of human endometrial tissue in a culture system mimicking the in vivo situation. In addition, they have shown that the active forms of collagenase 1 (MMP-1), of stromelysin 1 (MMP-3) and of gelatinase B (MMP-9) appear only just before and during menstruation in the human endometrium in vivo.

The endometrial tissue is in normal or healthy situations lining the uterine cavity. In the present invention it is described that cultures of human endometrial tissue serve as an

interesting model system to study the role and the function of MMPs in the degradation of the extracellular matrix. These tissue cultures can be kept in vitro for several days to mimick the in vivo situation: When cultured in the presence of physiological concentrations of ovarian steroids (estrogen and progesterone: EP), the tissue keeps it structural and functional characteristics. Depletion of EP for two days results in the lysis of the extracellular matrix, a situation which is identical to what occurs in vivo at the moment of menstruation.

According to a first embodiment, the invention relates to the use of at least one proteinase inhibitor which block the activity of selected matrix metalloproteinases or related proteinases for the preparation of a medicament for treating or preventing bleeding disorders of the endometrium. Preferably said proteinase inhibitor is a monoclonal antibody, a fragment thereof or a modified version thereof, directed against a metalloproteinase or related proteinase, preferably a matrix metalloproteinase. Preferably said monoclonal antibody is an inhibitory or blocking or inactivitating antibody, and said fragment or modified version of said antibody retains it inhibitory or blocking or inactivating effect.

The expression "bleeding disorders of the endometrium" as used herein relates to non-malignant disorders associated with abnormal bleeding of endometrial tissue, such as "abnormal uterine bleeding" or "endometriosis" or related diseases. The expression "bleeding disorders" and "diseases" can be used interchangeable.

Normal endometrial tissue lining the uterine cavity is indicated by the term "eutopic endometrium".

The expression "abnormal uterine bleeding" relates to excessive or prolonged menstrual bleeding occurring at the regular intervals of menstruation (menorrhagia), or to bleeding outside menstrual periods in the absence (metrorrhagia) or in the presence of hormonal treatment (breakthrough bleeding).

The term "endometriosis" relates to a condition in which tissue more or less perfectly resembling the uterine mucous membrane (the endometrium) and containing typical endometrial glandular and stromal elements occurs aberrantly in various locations preferentially in the pelvic cavity (ectopic endometrium) and generally is to be found in the region of the ovary, peritoneum, or recto-vaginal wall. However, it should be noted that ectopic endometrium tissue has been found in locations as disparate as the brain and lungs. Endometrial tissue may be determined histologically by looking for endometrial glands and stromal elements or by using markers.

The bleeding disorders of the endometrium can be observed with female primates, human as well as with some non-human primates.

Another embodiment of the invention relates to the use of at least one proteinase inhibitor for treating or preventing clinical disorders of the endometrium which are characterised by abnormal uterine bleeding, or for the preparation of a medicament for treating or preventing clinical disorders of the endometrium which are characterised by abnormal uterine bleeding.

Another embodiment of the invention is the use of at least one proteinase inhibitor for treating or preventing endometriosis or for the preparation of a medicament for treating or preventing endometriosis.

The invention further relates to any of the uses as described above wherein the proteinase inhibitor is an inhibitor of a tissue proteinase or an inhibitor of a metalloproteinase, preferably a matrix metalloproteinase inhibitor.

According to a furher embodiment the invention relates to the use of at least one proteinase inhibitor for treating or preventing bleeding disorders of the endometrium, said proteinase inhibitor preferably being an antibody directed against a matrix metalloproteinase, or a fragment or a modified version of such an antibody.

Several hundreds of human proteinases are known. They can be classified according to their localization or to their structure and catalytic mechanism.

Proteinases are also classified according to their catalytic mechanism into four major classes: serine-, cysteine-, aspartic proteinases and metalloproteinases.

Examples of interesting serine proteinases according to the invention are plasmin and the plasminogen activators. Examples of interesting cysteine proteinases according to the invention are lysosomal cysteine proteinases.

The term "tissue proteinases" comprises all proteinases except those of the digestive tract (stomach, intestine, ...) and exocrine secretions.

The expression "matrix metalloproteinases" refers to a family of enzymes which play a major role in extracellular matrix remodelling. The abbreviation "MMP" is frequently used throughout the present application to refer to matrix metalloproteinases.

Concerning the metalloproteinases, the invention relates to members of the family "matrix metalloproteinases" or MMPs, but also to the closely related family named ADAMs (a disintegrin and metalloproteinase). These ADAMs are included in the term "related

metalloproteinases". Examples of metalloproteinases according to the invention and explicitely claimed herein are the collagenases, the stromelysins and the gelatinases.

Examples of interesting matrix metalloproteinases according to the invention are collagenase 1 (MMP-1), stromelysin 1 (MMP-3), gelatinase A (MMP-2) and gelatinase B (MMP-9).

According to one embodiment of the invention, examples of such matrix metalloproteinase inhibitors are synthetic matrix metalloproteinase inhibitors. Selective or topical synthetic MMP inhibitors have been suggested in the art as a therapeutic prospect against abnormal endometrial bleeding, for instance in Marbaix et al. (1996, Proc. Natl. Acad. Sci. USA, 93, 9120-9125).

However, because antibodies can act more selective than synthetic inhibitors towards specific metalloproteinase, it is more interesting to use antibodies, for instance monoclonal antibodies, as specific and selective proteinase inhibitors in the present invention, Until now, no mention has been made towards the use of anti-MMP antibodies for treating or preventing abnormal uterine bleeding or endometriosis.

Accordingly, the invention relates to the use of at least one proteinase inhibitor, preferentially a matrix metalloproteinase inhibitor, for the preparation of a medicament for treating or preventing diseases associated with abnormal uterine bleeding or endometriosis, characterized in that said proteinase inhibitor is an antibody, a fragment thereof or a modified version thereof, which antibody or fragment or modified version thereof is selective for a specific matrix metalloproteinase.

The term "selective for a specific metalloproteinase" relates to an antibody, for instance a monoclonal antibody, a fragment thereof or a modified version thereof, which is specific for a selected metalloproteinase and not for another metalloproteinase. These antibodies, fragments thereof or modified versions thereof, can be specific by binding to any or a particular epitope on the selected metalloproteinase or can block the activity of said selected metalloproteinase, for instance by blocking the active site of the proteinase.

Several monoclonal antibodies against most metalloproteinases, including MMP-1, MMP-3 and MMP-9 blocking ones, are now commercially available, for instance from Calbiochem (see www.calbiochem.com), Chemicon (see www.chemicon.be) and Triple Point Biologics (see www.triplepoint-biologics.com). For instance, the Calbiochem catalogue describes numerous anti-MMP monoclonal antibodies, generated in Mouse, Guinea Pig, rabbit or sheep, which recognize the corresponding human matrix metalloproteinase. Most interesting

antibodies are those which inhibit, block or inactivate the matrix metalloproteinase, for instance the anti-MMP-9 antibody with Cat. NO IM09L. When no information is available on whether or not a commercial antibody is inhibitory or blocking or inactivating, this can be easily tested in an assay by investigating whether the said antibody is able to inhibit the proteinase activity of the corresponding MMP on its natural substrate. For instance, anti-MMP-1 antibodies can be analyzed for their inhibitory or blocking or inactivating action on the activity of MMP-1 on native collagen. This can be done using techniques well known by the skilled in the art.

According to a further embodiment, the invention relates to the use of monoclonal antibodies, fragments thereof or modified versions thereof, that block the activity of selected metalloproteinases for use in treating or preventing abnormal uterine bleeding or endometriosis, or for the preparation of a medicament for treating or preventing abnormal uterine bleeding or endometriosis.

According to another embodiment, said antibody is an inhibiting antibody, for instance an antibody which inhibits the binding of a selected metalloproteinase to components of the extracellular matrix.

The invention not only relates to the use of complete antibodies but also relates to the use of fragments of said antibodies or to modified versions of said antibodies for use in treating or preventing bleeding disorders of the endometrium, such as, but not restricted to, abnormal uterine bleeding and endometriosis, or for the preparation of a medicament for the treatment of said clinical disorders.

It should be clear that the antibody fragments and the modified versions thereof exhibit a similar biological effect as the antibody where it derived from, for instance having blocking or inhibiting activity.

According to the invention, the term "fragments of antibodies" relates to Fab and $F(ab')_2$ fragments, which are capable of binding to the antigenic determinant or epitope in question. Such fragments of antibodies thus bind to selected MMPs or block or inhibit their activity.

According to the invention, the term "modified versions of antibodies" relates to any antibody which is obtained or modified by human intervention. A specific way by which modified versions can be obtained is by recombinant DNA technology. To the group of "modified versions of antibodies" belong the following: recombinant antibodies, recombinant antibody fragments, single-chain antibodies, bispecific antibodies, diabodies and single-chain

diabodies, triabodies, intrabodies and all said modified versions of antibodies displayed on phages. An overview of methods used in antibody engineering is provided in several review articles (Carter, P. & Merchant, A.M. (1997) Curr. Opin. Biotechnol. 8, 449-454; Plückthun, A. & Pack, P. (1997) Immunotechnology 3, 83-105; Holliger, P. & Winter, G. (1997) Cancer Immunol. Immunother. 45,128-130) and on the "Antibody Engineering Page" (http://aximt1.imt.uni-marburg.de/~rek/AEPStart.html) at the internet. A particular group of modified versions of antibodies are humanized version.

The current invention also relates to the use of single chain fragments or humanized derivatives thereof because these antibody fragments do not induce a deleterious immunological response, and their small size favors tissue accessiblility. The present invention focuses further on the use of antibodies, fragments of antibodies or modified versions of antibodies that inhibit the enzymatic activity of MMP-1, MMP-3, MMP-9, or other MMPs or of related proteinases, implicated in the triggering of endometrial and endometriotic bleeding.

According to another embodiment, the invention relates to any of the above uses characterized in that said proteinase inhibitor is a monoclonal antibody, fragment thereof or a modified version thereof directed against human gelatinase B, obtainable by immunization of mice with human gelatinase B, fusion of their spleen cells with a myeloma cell line, expansion of the resulting culture and selection of individual clones.

According to another embodiment, the invention relates to any of the above uses characterized in that said proteinase inhibitor is a monoclonal antibody, fragment thereof or a modified version thereof directed against human collagenase1, obtainable by immunization of mice with human collagenase 1, fusion of their spleen cells with a myeloma cell line, expansion of the resulting culture and selection of individual clones.

According to another embodiment, the invention relates to any of the above uses characterized in that said proteinase inhibitor is a monoclonal antibody, fragment thereof or a modified version thereof directed against human stromelysin 1, obtainable by immunization of mice with human stromelysin 1, fusion of their spleen cells with a myeloma cell line, expansion of the resulting culture and selection of individual clones.

Antibodies directed against specific metalloproteinases can also be obtained by recombinant DNA technology, based on the amino acid information of existing monoclonal antibodies against metalloproteinases. For instance, PCT publication WO 99/25378 relates to synthetic antibodies, wherein the binding site of one antibody can be transplanted into a CDR

(complementarity determining region) of another immunoglobulin molecule to confer specificity against the antigen recognized by the first antibody. For example, modified antibodies containing the variable domain sequences from MMP-1, MMP-3 or MMP-9 antibodies can be constructed.

Antibodies which inhibit or inactivate or block specific MMP's can also be generated using phage-display technology. A review on the antibody phage display technology and its applications is given by Hoogenboom et al. in: Immunotechnology, 1998, volume 4, pages 1-20. Therefore, it is also an embodiment of the invention to use recombinant antibodies which specifically inhibiting or inactivating or blocking MMP's and which are generated using the well known techniques of antibody phage-display.

According to another embodiment the invention relates to the uses of single-chain fragments of the above described antibodies, where the VH and VL domain of an antibody are linked by flexible spacer. Instead of joining these domains with a peptide linker, the VH and VL domain can be also linked covalently by a disulfide bridge by genetically engineering cysteine residues at the VH-VL interface at positions allowing the formation of a disulfide bond generating disulfide-stabilized Fv fragments (dsFv). The single-chain fragments are obtainable by, for instance recombinant DNA techniques.

The present invention further relates to any of the uses above described wherein said protease inhibitor is an antibody, a fragment thereof or a modified version thereof, that specifically inhibits or inactivates the matrix proteinase MMP-9.

One example of such an antibody is a mouse monoclonal antibody (3G12mAB) that selectively blocks the activity of human MMP-9, has been produced and characterized in Paemen et al., 1995 (Eur. J. Biochem. 234, 759-765).

The 3G12mAb antibody:

i) blocks the activity of MMP-9 without affecting its closest family member, MMP-2 (gelatinase A) or other MMPs;

ii) has been tested in vivo in Rhesus Monkeys showing inhibitory activity on the MMP-9 dependent recruitment of immune cells, but no toxicity.

A recombinant active single-chain fragment (scFv) of this antibody, named 3G12-scFv has been developed, is six times smaller than the 3G12mAB, but has the same affinity for MMP-9 and is not immunogenic. The construction of this scFv is described in Zhou et al. (1997) FEBS letters 414, 562-566.

The present invention therefore relates to the use of an anti-MMP9 antibody, such as the known antibody 3G12mAB, or a single-chain fragment thereof for the preparation of a medicament for treating or preventing clinical disorders of the endometrium, such as abnormal uterine bleeding and endometriosis.

Another embodiment of the present invention is thus the use of an inhibitory or blocking or inactivitating antibody, a fragment thereof or a modified version thereof, for instance a single-chain fragment, for the preparation of a medicament against abnormal uterine bleeding and endometriosis, preferably, said antibody is specific for the selected MMP-1, MMP-3, MMP-9 or other MMPs or specific for another related metalloproteinase.

Other interesting antibodies or modified antibodies according to the invention are bispecific antibodies. Several forms of bispecific antibodies methods for their recombinant production are known in the art. One form of a bispecific antibody may be a bivalent diabody.

The present inventors for instance demonstrated that in short-term cultures of human endometrial tissue explants, the expression and the activation of proMMP-9 is inhibited by progesterone. An addition of only 10 nM of progesterone seems to be sufficient to completely block the activation of proMMP-9. The present inventors have shown that, in vivo, proMMP-9 is only activated at the start of the menstruation, and that MMP-3 is responsible for this activation ex vivo.

Therefore, the invention also relates to the use of a bispecific antibody or a bivalent diabody for the preparation of a medicament for treating or preventing clinical disorders of the endometrium, said antibody or diabody being bi-specific for two selected matrix metalloproteinases. According to one embodiment of the invention, the bispecific antibody is selected for MMP-9 and MMP-3.

Bispecific antibodies combine antigen-binding sites against two different antigens. Various methods can be applied to generate bispecific antibodies, including chemical cross-linking and hybrid hybridoma techniques. Various strategies are available to generate bivalent or bispecific recombinant antibody fragments. Two identical or different scFv fragments can be combined by introducing an additional linker between the C-terminus of the first scFv and the N-terminus of the second scFv ((scFv)2 fragments or scFv tandems). Similarly, it should be possible to join two camelized VH domains with a flexible linker. Alternatively, cysteine residues can be introduced at the C-terminus of an scFv resulting in disulfide-crosslinking of two scFv.

Diabodies represent a totally different approach. Here, the linker between the VH and the VL domain is too short (normally 0 - 5 amino acids) to allow assembly of the VH and VL domains of one chain. This leads to the assembly of a dimeric molecule (diabody) where the VH and VL domain of two different chains form a double-headed molecule with the two binding sites pointing away from each other. Using two different antibody specificities (A and B) expressed in the format VHA-VLB and VHB-VLA in the same cell, bispecific diabodies are formed.

Another embodiment of the invention relates to the use of at least one metalloproteinase or matrix metalloproteinase inhibitor, or an inhibitor of a related metalloproteinase for treating abnormal uterine bleeding or endometriosis.

The invention further relates to a pharmaceutical composition comprising any of the above described proteinase inhibitors and a suitable carrier or excipient.
More specific the invention relates to a medicament for treatment or prevention of abnormal uterine bleeding or endometriosis comprising an anti- MMP antibody in an effective amount for inhibiting lysis of the extracellular matrix of the endometrium.

A further embodiment of the invention relates to a method of treatment or prevention of abnormal uterine bleeding or endometriosis comprising administering to a mammal in need of such treatment or prevention a therapeutically effective amount of an inhibitor of a matrix metalloproteinase inhibitor, characterized in that said inhibitor is a monoclonal antibody, a fragment thereof or a derivative thereof, able to inhibit the interaction of said matrix metalloproteinase with the extracellular matrix of the endometrium. Preferably said monoclonal antibody is directed against a matrix metalloproteinase selected from the group of collagenase 1 (MMP-1), stromelysin 1 (MMP-3) and gelatinase B (MMP-9). Preferably said mammal is human or a non-human primate.

The anti-MMP antibodies can be administered to a patient in need thereof in several ways and in different amounts depending on the way of administration. For instance, the antibody can be mixed with a suitable pharmacologically acceptable exipient and administered in amounts ranging from 1 mg to 0.01 mg per kilogram of bodyweight, preferably in amounts ranging from 0.5 to 0.05 mg or from 0.2 to 0.1 mg per kilogram of bodyweight. When administered intravenously, amounts ranging from 10 to 1 mg per kilogram of bodyweight, preferably in amounts from 7 to 3 mg per kilogram of bodyweight.

When administered intra-uterine, amounts ranging from 5 to 0.01 mg can be used, preferably amounts ranging from 2 to 0.05 mg.

When administered subcutaneously, amounts ranging from 2 to 0.02 mg per kilogram of bodyweight can be used, preferably amounts ranging from 1 to 0.5 mg per kilogram of bodyweight.

The antibody or the composition comprising the antibody may be administered in a single doses or may be administered in several identical or smaller doses distributed over a time period of several days. Furthermore, the treatment with the anti-MMP antibody can be combined with other treatment, for instance ovarian steroids or another medicament, further enhancing the effect of the anti-MMP antibody.

One preferred way of administration is intra-uterine, for instance as a medicament released from an IUD (intra uterine device). Such a way of administration would require much lower doses, and are not dependent on body weight, for instance a single intra-uterine administration of 1 to 5 mg can be used, or repeated administration of 0.01 to 1 mg per day.

It should be clear that the use of antibody fragments, or mini-antibodies, or other smaller variants of antibodies as described earlier, also would require lower doses, the required dose being inversely proportional to their molecular mass.

The invention now being generally described may be more clearly understood by reference to the following examples, which are included merely for purposes of illustration of certain aspects and embodiments of the present invention and are not intended to limit the invention.

BRIEF DESCRIPTION OF FIGURE

Figure 1. Silverstaining of fibers in extracellular matrix

After 3 days of culture in the absence of both ovarian steroids (EP) and antibodies (Fig. 1.1), the silver-stained extracellular fibers have disappeared, in contrast with Fig 1.2 (culture with EP) and Fig 1.3 (culture with an inhibitory anti-MMP-3 antibody). The addition of a non-inhibitory anti-MMP-1 (Fig 1.4) did not inhibit lysis of the fibers.

EXAMPLES

Example 1: Preparation and characterization of monoclonal antibodies against proteinases

Human proteinases, for instance matrix metalloproteinases, are purified according to standard protocols available in the art. For instance gelatinase B (MMP-9) is purified from neutrophils is described at page 760 of Paemen et al. (1995), Eur. J. Biochem. 234, 759-765. Standard protocols are used for production of monoclonal antibodies in mice against human matrix metalloproteinases. For instance, production, screening and characterization of anti-gelatinase B (MMP-9) producing hybridomas is described at pages 760-761 of Paemen et al. (1995), Eur. J. Biochem. 234, 759-765.

Briefly, mice such as Balb/c mice are immunized with a purified proteinase preparation (e.g. 10 µg/mouse in complete Freund's adjuvant) and boosted twice, each time with a few weeks interval. A few days after a final booster is given, the spleens of the mice are removed. Spleen cells are fused with myeloma cells. The resulting hybridomas are cloned in 96-well microtiter plates and, after screening, the positive lines are expanded and cloned by limiting dilution. After cloning, the hybridoma cells are screened in different assays for specific binding to the selected proteinase. Clones from positive wells can be subjected to several rounds of recloning to yield several isolated and pure monoclonal antibody cell lines.

Monoclonal anti-MMPs are also prepared by injecting a synthetic oligopeptide corresponding to a specific known sequence of the MMP. The choice of the sequence can take into account the 3-D structure of the enzyme, and will thus determine the localization of the epitope in the native enzyme and the inhibitory effect of the antibody.

Isolated monoclonal antibodies are further characterized for their specificity, immunoreactive properties, binding affinity and cross-reactive properties in standard assays.

Each of the isolated monoclonal antibodies is individually tested for its ability to specifically block the activity of the selected proteinase or matrix metalloproteinase against which it was raised. For each of the selected proteinases, specific tests can be set up depending on its specificity.

Well-performing monoclonal antibodies are selected for further use.

Blocking antibodies are identified according to commonly known methods.

Example 2: Cloning and expression of single-chain immunoglobulin variable fragments

The variable regions of the light and heavy chain genes of interesting blocking or inhibiting monoclonal antibodies, selected in Example 1, are isolated by RT-PCR, cloned and sequenced using standard protocols known in the art. Subsequently, the VH and VK genes are cloned in an expression vector for *E.coli.* Such vectors or expression systems are commercially available, for instance the pCANTAB expression system of Pharmacia.

After expression in E.coli, the scFv are affinity-purified and further characterized.

The cloning and expression in Escherichia coli of a human gelatinase B-inhibitory single-chain immunoglobulin variable fragment (scFv) is described in Zhou et al. (1997) FEBS Letters 414, 562-566.

Example 3: Model system for selecting protease inhibitors in a human endometrial tissue culture

An *in vitro* model system comprising a human endometrial tissue can be kept in culture for several days exhibiting similar structural and physiological characteristics as the *in vivo* situation by supplementation of physiological concentrations of ovarian steroids (1 nM estrogen and 100 nM progesterone: EP). Depletion of said steroids for two or three days induces the lysis of the extracellular matrix, as happens in vivo at the onset of menstruation.

Example 4: Assays for testing the activity of anti-MMP antibodies in a human endometrial tissue culture model system

The effect of inhibitory or blocking antibodies raised against matrix metalloproteinases, such as MMP-1, MMP-3 and MMP-9, were studied on cultured human endometrial explants in the absence of EP for two or three days. At the same time, structural (classical histology, silver-staining, immunodetection of collagen I, III and IV) and biochemical (expression and activation of proMMP; quantitative zymography) modifications were studied. Each of the test conditions (- EP; - EP + control antibody; - EP + anti-MMP antibody, + EP - antibody) was performed in quadruplicate. The ovarian steroids were added as water-soluble complexes with 2-hydroxypropyl-beta-cyclodextrin (Sigma-Aldrich, Bornem, Belgium). The antbodies were solubilized in culture medium. When the term "vehicle", is used, this means 2-hydroxypropyl-beta-cyclodextrin + culture medium.

These tests were repeated with endometrial explants from several patients at different phases of the menstrual cycle. Also fragments and modified versions of said antibodies, and bispecific antibodies and diabodies are included in these tests.

Explants of a human mid-secretory endometrium were cultured as described (Marbaix et al., 1995, Biochem. J. 305, 1027-1030) for 3 days in the presence of culture medium supplemented with the following agents:

1) only vehicle
2) 1 nM estradiol and 100 nM progesterone
3) 10 µg/ml monoclonal anti-MMP-3 inhibitory antibody (IgG1, clone 55-2A4, Cat. Number IM36L of Calbiochem, La Jolla, CA, USA)
4) 10 µg/ml monoclonal anti-MMP-1 non-inhibitory antibody (IgG1, clone III7, Cat. Nmber IM67 of Calbiochem, La Jolla, CA, USA)

After 3 days of culture, the fibrillar extracellular network (the so-called reticulin fibers) of the explants has been analyzed by silverstaining as described (Marbaix et al., 1996, Proc. Natl. Acad. Sci. USA, 93, 9120-9125) and semi-quantitatively evaluated by 6 persons who were not aware of the code. The disappearance of the reticulin fibers (illustrated on Figure 1) was scored from 0 (full preservation) to 4 (complete breakdown):

1) 4
2) 0
3) 0
4) 3

These data show that an anti-MMP-3 inhibitory antibody blocked the degradation of the extracellular fibrillar network, as efficiently as the combined two ovarian steroids.

A similar experiment using an anti-MMP-9 inhibitory antibody (IgG1, clone 56-2A4, Cat. Number IM37L of Calbiochem, La Jolla, CA, USA) resulted in a partial but significant inhibition of the degradation of the extracellular fibrillar network.

Example 5: Effects of anti-MMP antibodies after 18 hours pre-culture in the presence of ovarian steroids

In order to ensure complete diffusion of the antibodies in the endometrial explants before the onset of their degradation induced by depletion of EP, explants of a human secretory

endometrium were first cultured as described above for 18 hours in the presence of culture medium supplemented with EP and with the following agents :

1) vehicle
2) vehicle
3) a control monoclonal IgG
4) a monoclonal IgG anti-MMP-1

After 18 hours of culture, the culture media were replaced by the same fresh media deprived of EP except in condition 2, wherein EP were added, serving as positive control. After one additional day of culture, media were replaced by the same respective fresh media. After one more day, the explants were analyzed as described. The disappearance of the reticulin fibers was scored from 0 (full preservation) to 4 (complete breakdown) :

1) 3
2) 1
3) 3
4) 1

These data show that a monoclonal anti-MMP-1 antibody inhibited the degradation of the extracellular fibrillar network (e.g. condition 4), as efficiently as the combined two ovarian steroids (e.g. condition 2).

Example 6: Diffusibility of the antibodies into cultured endometrial explants

Using polyclonal anti-type III collagen immunoglobulins at various concentrations (0, 1, 2.5 and 10 microg /ml), we have observed staining of type III collagen fibers throughout the explants after 20 hours of culture in the presence of 10 microg /ml, but only peripheral staining at 2.5 microg /ml. This demonstates that full-size (160.000 kD) immunoglobulins are able to diffuse into cultured endometrial tissue fragments.

Example 7: *In vitro* experiments

One antibody, or a combination of antibodies, which is (are) effective in *in vitro* blocking endometrial tissue breakdown in the culture system as described in the previous examples, are tested *in vivo* on a menstruating non-human primate and later on female human volunteers.

CLAIMS

1. Use of at least one proteinase inhibitor for the preparation of a medicament for treating or preventing abnormal uterine bleeding or endometriosis, characterized in that said proteinase inhibitor is a monoclonal antibody, a fragment thereof or a modified version thereof, directed against a metalloproteinase.
2. Use according to claim 1 wherein said metalloproteinase is a matrix metalloproteinase selected from the group comprising collagenases, stromelysins and gelatinases, or a related metalloproteinase.
3. Use according to claim 1 or 2 wherein said metalloproteinase is selected from collagenase 1 (MMP-1), stromelysin 1 (MMP-3) and gelatinase B (MMP-9).
4. Use according to any of claims 1 to 3 characterized in that said proteinase inhibitor is a monoclonal antibody, a fragment thereof or a modified version thereof, directed against human gelatinase B, obtainable by immunization of mice with human gelatinase B, fusion of their spleen cells with a myeloma cell line, expansion of the resulting culture and selection of individual clones.
5. Use according to any of claims 1 to 3 characterized in that said proteinase inhibitor is a monoclonal antibody, a fragment thereof or a modified version thereof directed against human collagenase 1, obtainable by immunization of mice with human collagenase 1, fusion of their spleen cells with a myeloma cell line, expansion of the resulting culture and selection of individual clones.
6. Use according to any of claims 1 to 3 characterized in that said proteinase inhibitor is a monoclonal antibody, a fragment thereof or a modified version thereof directed against human stromelysin 1, obtainable by immunization of mice with human stromelysin 1, fusion of their spleen cells with a myeloma cell line, expansion of the resulting culture and selection of individual clones.
7. Use according to any of claims 1 to 6 wherein said proteinase inhibitor is a recombinant single-chain fragment.
8. Use according to any of claims 1 to 4 wherein said proteinase inhibitor is a recombinant single-chain fragment derived from a blocking or inactivating antibody of gelatinase B.
9. Use of a monoclonal antibody, a fragment thereof or a modified version thereof, directed against human gelatinase B for the preparation of a medicament for treating abnormal uterine bleeding or endometriosis.

10. Use of a monoclonal antibody, a fragment thereof or a modified version thereof, directed against human stromelysin 1 for the preparation of a medicament for treating abnormal uterine bleeding or endometriosis.
11. Use of a monoclonal antibody, a fragment thereof or a modified version thereof, directed against human collagenase 1 for the preparation of a medicament for treating abnormal uterine bleeding or endometriosis.
12. Use of at least one proteinase inhibitor for treating or preventing abnormal uterine bleeding or endometriosis, characterized in that said proteinase inhibitor is a monoclonal antibody, a fragment thereof or a modified version thereof, directed against a metalloproteinase.
13. Use according to claim 12 wherein said wherein said metalloproteinase is selected from collagenase 1 (MMP-1), stromelysin 1 (MMP-3) and gelatinase B (MMP-9).
14. Use of at least one monoclonal antibody, a fragment thereof or a modified version thereof, directed against gelatinase B (MMP-9) for treating or preventing abnormal uterine bleeding or endometriosis.
15. Use of at least one monoclonal antibody, a fragment thereof or a modified version thereof, directed against stromelysin 1 (MMP-3) for treating or preventing abnormal uterine bleeding or endometriosis.
16. Use of at least one monoclonal antibody, a fragment thereof or a modified version thereof, directed against collagenase 1 (MMP-1) for treating or preventing abnormal uterine bleeding or endometriosis
17. Pharmaceutical composition comprising a proteinase inhibitor as defined in any of claims 1 to 12 and a suitable acceptable carrier or excipient.
18. Method of treatment or prevention of abnormal uterine bleeding or endometriosis comprising administering to a mammal in need of such treatment or prevention a therapeutically effective amount of an inhibitor of a matrix metalloproteinase, characterized in that said inhibitor is a monoclonal antibody, a fragment thereof or a derivative thereof, able to inhibit the interaction of said matrix metalloproteinase with the extracellular matrix of the endometrium.
19. Method according to claim 18, wherein said monoclonal antibody is directed against a matrix metalloproteinase selected from the group of collagenase 1 (MMP-1), stromelysin 1 (MMP-3) and gelatinase B (MMP-9).

20. A medicament for treatment or prevention of abnormal uterine bleeding or endometriosis comprising an anti- MMP antibody in an effective amount for inhibiting lysis of the extracellular matrix of the endometrium.

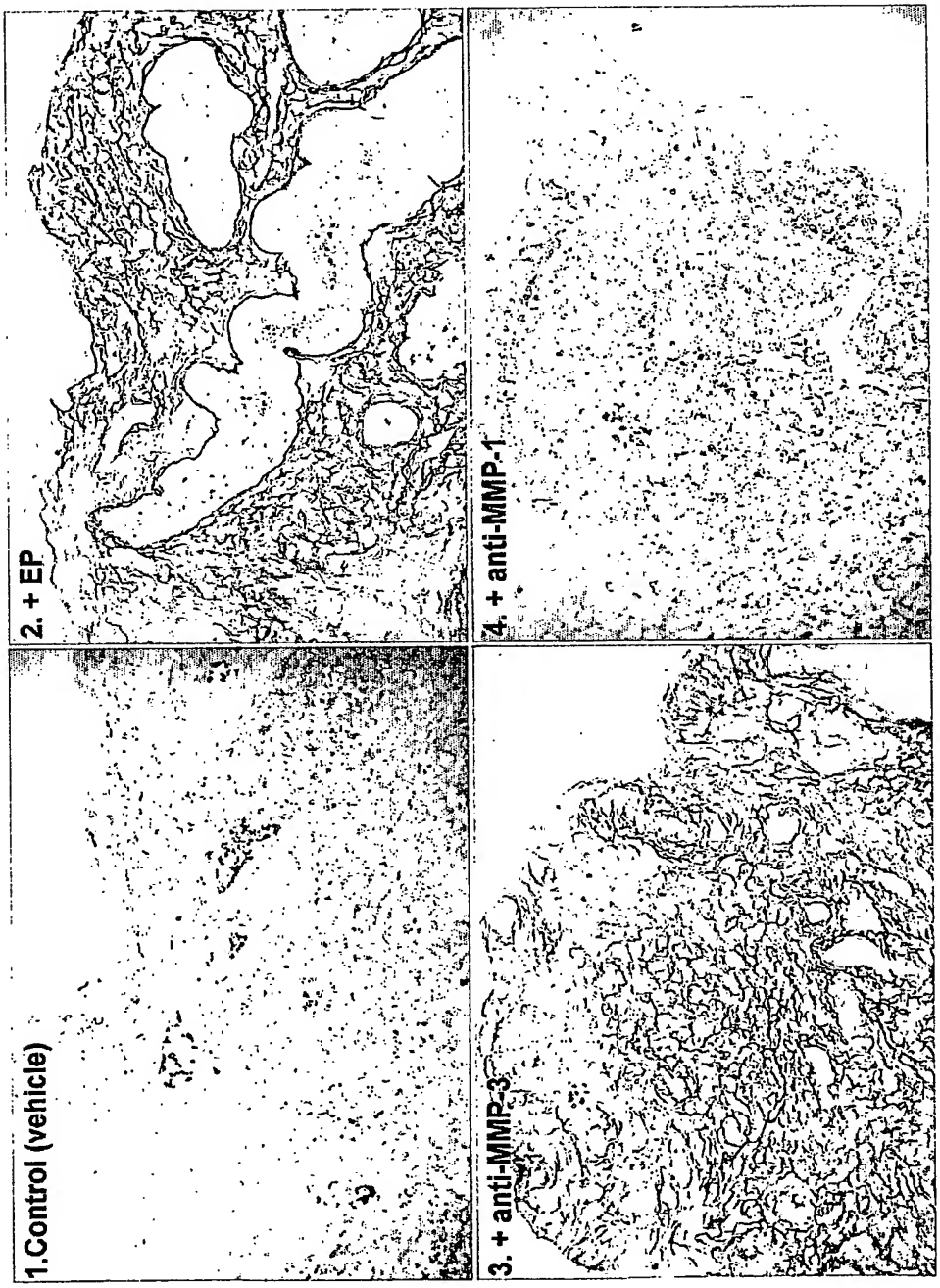

Figure 1

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 May 2003 (30.05.2003)

PCT

(10) International Publication Number
WO 2003/044058 A3

(51) International Patent Classification[7]: A61K 39/395, A61P 15/00

(21) International Application Number: PCT/EP2002/013074

(22) International Filing Date: 21 November 2002 (21.11.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
01870260.5 23 November 2001 (23.11.2001) EP

(71) Applicant *(for all designated States except US)*: **UNIVERSITE CATHOLIQUE DE LOUVAIN** [BE/BE]; 1 Place de l'Université, B-1348 Louvain-la-Neuve (BE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **EECKHOUT, Yves, Joseph** [BE/BE]; 38 avenue de l'Espinette, B-1348 Louvain-la-Neuve (BE). **COURTOY, Pierre, Joseph** [BE/BE]; 4 rue Pierre Delacroix, B-1150 Bruxelles (BE). **MARBAIX, Etienne, Michel** [BE/BE]; 2 Clos du Nysdam, B-1310 La Hulpe (BE).

(74) Agents: **BRANTS, Johan, Philippe, Emile** et al.; De Clercq, Brants & Partners, E. Gevaertdreef 10 a, B-9830 Sint-Martens-Latem (BE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report: 22 January 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: MEDICAL USE OF ANTIBODIES DIRECTED AGAINST HUMAN MATRIX METALLOPROTEINASES OR RELATED TISSUE PROTEINASES FOR THE TREATMENT OF ABNORMAL UTERINE BLEEDING AND ENDOMETRIOSIS

(57) Abstract: The invention relates to the field of metalloproteinases and their involvement in abnormal uterine bleeding or in endometriosis. The invention provides inhibitors of tissue proteinases or of metalloproteinases for preparing medicaments for treating or preventing bleeding disorders of the endometrium. More specific, these inhibitors are inhibitory antibodies against matrix metalloproteinases.

WO 2003/044058 A3

INTERNATIONAL SEARCH REPORT

Internat application No
PCT/EP 02/13074

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/395 A61P15/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PRUIJT-JFM ET AL.: "Prevention of interleukin-8-induced mobilization of hematopoietic progenitor cells in rhesus monkeys by inhibitory antibodies against the metalloproteinase gelatinase B (MMP-9)" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 96, September 1999 (1999-09), pages 10863-10868, XP002192381	17,20
Y	abstract page 10863, right-hand column, paragraph 2	1-20

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 July 2003	07/08/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lechner, O

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internati \pplication No
PCT/EP 02/13074

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with Indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 872 146 A (BAXTER ANDREW DOUGLAS ET AL) 16 February 1999 (1999-02-16) column 1, paragraphs 2,3 column 6, line 52-67 column 7, line 1-30 claims 1,14,22	1-20
Y	MARBAIX-E ET AL.: "Menstrual breakdown of human endometrium can be mimicked in vitro and is selectively and reversibly blocked by inhibitors of matrix metalloproteinases" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 93, 1996, pages 9120-9125, XP002192382 cited in the application page 9120, right-hand column, paragraph 3 page 9124, right-hand column, paragraphs 1,2 page 9125, left-hand column, paragraph 2	1-20
A	MATRISIAN-LM ET AL.: "Metalloproteinase expression and hormonal regulation during tissue remodelling in the cycling human endometrium" CONTRIBUTIONS TO NEPHROLOGY, vol. 107, 1994, pages 94-100, XP001063900 the whole document	1-20

INTERNATIONAL SEARCH REPORT

Int ial application No.
PCT/EP 02/13074

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 12-16, 18 and 19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 2, 4-6, 9-16 and 18-20 (only partially)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 2, 4-6, 9-16 and 18-20 (only partially)

Present claims 18-20 relate to antibodies and fragments thereof defined by reference to a desirable characteristic or property, namely the ability to inhibit the interaction of a matrix metalloproteinase with the extracellular matrix of the endometrium and for inhibiting lysis of the extracellular matrix of the endometrium.

Claims 4-6, 9-16, 18 and 19 also relate to an extremely large number of possible derivatives and modified versions.

Finally, claim 2 relates to an extremely large number of not clearly defined "related metalloproteinases".

The claims cover all antibodies/derivatives/modified versions/related metalloproteinases having these characteristics or properties and exhibiting "similar" biological effects (c.f. page 5, line 22-24 as well as line 28-30), whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such antibodies. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the antibodies by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the use of anti-MMP antibodies and fragments thereof for the preparation of medicaments for treating abnormal uterine bleeding or endometriosis.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internati pplication No
PCT/EP 02/13074

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5872146 A	16-02-1999	AU 720239 B2	25-05-2000
		AU 2302697 A	29-10-1997
		CA 2248082 A1	16-10-1997
		EP 0891375 A1	20-01-1999
		WO 9738007 A1	16-10-1997
		JP 2000510103 T	08-08-2000
		ZA 9702895 A	06-04-1998